EVENT ABSTRACT

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BONE REMODELLING STUDY USING STRONTIUM ENRICHED HYDROXYAPATITE NANOPARTICLES

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INTRODUCTION:

Microgravity (MG) significantly modifies the metabolism of bone leading to site-specific alterations in remodeling of the bone tissue. A decrement in bone formation and an increase in bone resorption determine a significant loss of bone mass causing bone fragility and therefore a greater risk of fractures. The proposed study is focused on the development of the countermeasures to be taken in order to reduce the process of bone demineralization, while promoting a greater deposition of bone matrix by using a nanotherapeutic approach. Strontium (Sr) is present in the mineral phase of bone, in particular in regions with high metabolic activity turnover. Recently, both in vitro and in vivo studies of Sr effects showed the reduction of bone resorption and the promotion of bone formation. The mineralization process is crucial to the load-bearing characteristics of the bone extracellular matrix. In a previous study (Frasinelli et al., 2017), we produced stable and biocompatible suspensions of calcium (Ca100) and Strontium (Sr100) hydroxyapatite nanoparticles (nHAps) to be potentially used to deliver Ca or Sr to bone cells. In the work presented here, we have studied the role exerted by the addition of exogenous Ca100-nHAp or Sr100-nHAp as countermeasure to MG induced osteoporosis, by using a model of human bone marrow mesenchymal stem cells (hBMSCs) differentiated in simulated MG (Random Positioning Machine, RPM). Further, to deeper investigate the mechanism on bone formation, we studied the spatiotemporal dynamics of mineral deposition by hBMSCs differentiating toward osteoblasts promoted by the presence of exogenous hydroxyapatite nanoparticles, using scanning micro X-ray diffraction and scanning micro X-ray fluorescence (Campi et al., 2017).

MATERIALS AND METHODS:

Human bone marrow mesenchymal stem cells were differentiated for 8 or 28 days as previously described (Campi et al., 2017) but cultured in simulated MG using a RPM (Fokker space) at 37°C. Ground controls (GC ctrl) were performed in the same room on the RPM frame to simulate the effect of instrument vibration (Fig. 1 blue line). Cells were untreated (RPM ctrl, red line) or treated with Ca100-nHAp (RPM Ca100, Fig. 1 green line) or Sr100-nHAp (RPM Sr100, Fig. 1 yellow line) produced and characterized as previously described (Frasinelli et al., 2017). At 28 days of cell differentiation, samples were analyzed for bone markers using confocal laser scanning microscopy (CLSM) and Elisa techniques.

Moreover, samples were fixed to be further analyzed at the European Synchrotron Radiation Facility (ESRF) in Grenoble, (France) using scanning micro X-ray diffraction and scanning micro X-ray fluorescence.

RESULTS

Simulated MG strongly affects hBMSCs differentiation at day 28; bone markers are strongly modified in RPM exposed cells with a reduction of crystal size, a decrease in calcium deposition measured by alizarin Red, collagen 1, osteocalcin and ALP expression. Data obtained in our study are resumed in figure 1 and expressed as percent of control: in comparison with RPM control cells (red line), the RPM samples treated with Ca100-nHAps (green line) or Sr100-nHAps (yellow line) were able to strongly revert the effect of MG. In particular, this effect was higher in the presence of Sr100-nHAps in comparison to Ca100-nHAps.

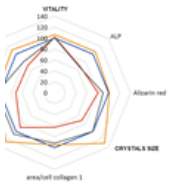
At the molecular level, the added nanoparticles positively modulated the expression of bone-specific markers and enhanced calcified matrix deposition during osteogenic differentiation when analyzed both at early and late stages of differentiation (8 and 28 days), increasing matrix protein deposition in comparison with 1 g treated samples. The nucleation, growth and spatial arrangement of newly deposited hydroxyapatite nanocrystals were evaluated using scanning micro X-ray diffraction and scanning micro X-ray fluorescence (data not shown). As leading results, we have found the emergence of a complex scenario where the spatial organization and temporal evolution of the process exhibit heterogeneous and self-organizing dynamics.

CONCLUSIONS

The possibility of controlling the differentiation kinetics, through the addition of synthetic nanoparticles, paves the way to empower the generation of more structured bone scaffolds in tissue engineering and to design new drugs in regenerative medicine, useful during long term space flights.

Figure 1 - Effects of simulated MG (28 days) on hBMSCs untreated or treated with Ca and Sr enriched nHAPs. Bone markers expression (ALP, alkaline phosphatase activity, Ca crystals size, Collagen area and florescence intensity, Osteocalcin area and fluorescence intensity) is shown to be reverted by the addition in the culture media of Ca100-nHAps (green line) or, at higher extent, by Sr100-nHAps (yellow line), in comparison with untreated cells (red line). In blue line are indicated the cells cultivated in ground control, not exposed to RPM.

Figure 1



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